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Comparison of serum steroidal hormone concentrations in buller steers, riders, and uninterested penmates : implication for the etiology of the buller steer syndrome in North American feedlots

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Comparison of serum steroidal hormone concentrations in buller steers, riders, and uninterested penmates: implication for the etiology of the buller steer syndrome in North American feedlots

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CHAPTER 1. GENERAL INTRODUCTION

Thesis Organization

The first chapter reviews the potential causes of the buller steer syndrome. The second chapter is an independent manuscript that reports a study performed to determine whether variations in serum hormone concentrations are associated with the buller steer syndrome. The final chapter summarizes and discusses the conclusions drawn from the review and research. A reference list is included at the end of each chapter.

Introduction

According to the National Animal Health Monitoring System (NAHMS) the number of feedlot cattle on feed in the United States increased 12.6 % from 1995 through 2000. There were 13,983,000 head of cattle on feed during the year of 2000. Approximately one-half (53 %) of all placements were beef and beef crossbred steers and heifers weighing > 700 pounds at placement. Seventy-two percent of the total cattle on feed are placed into feedlots located in Colorado, Iowa, Kansas, Nebraska, and Texas.[1]

The average incidence reported for buller steers in a feedlot population is 2-3 % of the steer population (range 0-11.2 %), and the case fatality rate may exceed 1 %.[2-4] A survey ranked the buller steer syndrome third behind bovine respiratory disease and foot rot as the most costly diseases in North American feedlots.[4] It is estimated that buller steers cost the cattle industry $70 per head. Death loss, carcass condemnations, decreased live weight gain, and treatments of injury cause economic loss.

Buller steers have been classified as either type I or type II.[5] Type I buller steers are considered the "true buller." These steers assume a stance similar to that of pubertal heifers

in estrus. It is not uncommon for these steers to be ridden and harassed to the point of collapsing. Type II buller steers are considered steers of "unfair social circumstances." These buller steers will not assume an estrus like stance.[5] The type II buller steer will use aggressive acts such as head butting to ward off the group of riders. Eventually the buller steer succumbs to the harassment and lies down, however, the riders will continue their activities on the downed buller steer.[5]

Literature Review

Time of occurrence in the feeding period

Taylor and colleagues documented the distribution of bullers by days on feed, and the highest incidence occurred within the first 30 days on feed.[4] Table 1 demonstrates the number of bullers identified during 30-day increments with day 0 as the day of arrival in the feedlot. The growth hormone implant used in these cattle contained 20 mg estradiol benzoate and 200 mg progesterone (Synovex S®, Fort Dodge Animal Health, Overland Park, KS).

Table 1. The Number of Bullers Identified by DOF within Management Group (Data adapted from Taylor et al. 1997)

DOF	1991 FSC	1992 YS	1992 FSC	1993 WCS	1993 YS
0-29	201	111	307	40	477
30-59	161	92	74	9	113
60-89	98	40	41	5	56
90-119	72	39	26	10	31
120-149	39	26	7	8	7
150-179	16	20	0	0	0
180-209	7	6	0	0	0
Total head	19,170	18,417	19,257	4507	17,094

DOF = Days on Feed, FSC = Fall steer calves, YS = Yearling steers, WSC = Winter steer calves

The median days on feed (DOF) for buller identification in the fall steer calf (FSC) group was 45 days, 21 days for the winter steer calf (WSC) group, and 15 days for the yearling steer (YS) group. The relapse rate after 3 days in the hospital averaged 30%. Irwin and colleagues reviewed 409 buller steers and found the mean time for bulling activity after arrival in the feedyard was 61.4 days with a range for bulling activity of 1-221 days.[6] Therefore, buller steers are more likely to be observed during the first third (< 60 days) of the feeding period, and the factors that have been implicated as causing the buller syndrome may predominate during this period.

Seasonal effects

The seasonal occurrence of buller steers varies in different regions of North America. In a Texas study, buller steers were found most often between the months of November and December.[6] Researchers in Kansas found the most activity in July and August.[2] A Colorado study found peak bulling activity occurred during the summer and early fall, although this was during a time when cattle were being fed a ration containing freshly chopped alfalfa, which might have contained high levels of estrogen.[7] The general trend in the literature agrees with our observation in Iowa of increased buller activity starting in August and continuing through late Fall.

Pen size and density

The relationship of group size and amount of pen space per animal to incidence of buller steers has been examined in a study observing 11,000 steers over three years. It was determined that the amount of pen space available per steer or steer weight at the time of bulling activity had no influence on the number of buller steers. The range of steers per pen in that study was 70 to 416 and the pen space per head was 7.6 to 32.5 m^2.[2]

Records from over 19,000 steers were reviewed in an unrelated study.[8] A relationship between pen space and buller steer occurrence was not found. In contrast, Irwin and colleagues found a direct correlation between the number of steers in the pen and the occurrence of buller steers.[6] The average number of steers per pen was 204 with a range of 52-466. A relationship between buller occurrence and pen size, square meters per animal, or entry weight was not found.

Effect of mixing and age of steers

Mixing steers from multiple sources may contribute to bulling activity. An increased occurrence in the mounting activity in pens of newly introduced cattle suggests that development of social hierarchy may be a significant factor causing buller steers.[9] Klemm and colleagues observed the effects of adding 50 non-bullers to a pen of steers. During the first 6 hours there was an initial high rate of bulling followed by a marked decline by 24 hours post addition. Addition of a second group of 50 non-bullers had no effect. After the third addition of 50 non-bullers there was an increase in buller activity for 24-36 hours post additon.[10]

Lott and colleagues suggest bullers occur significantly sooner in older cattle compared to younger cattle after mixing.[3] Taylor and colleagues also noted over a 2-year study that the highest incidence of bullers occurred in groups of yearlings, compared to calves. Tennessen and colleagues grouped steers and bulls (separated by sex) into pens of 8 animals.[11] Individual groups were mixed so that each group was exposed to 6 new pen mates at 9, 12, and 15 months of age. They found more sexual investigations in the bull group compared to the steer group, and aggressive behavior in both groups was non-existent by 10 days post mixing.

Concurrent diseases

Concurrent diseases have been implicated as a reason certain steers are harassed by their pen mates. Brower and colleagues suggest many bullers have accompanying conditions such as pneumonia or urinary calculi when removed from the pen. A plausible hypothesis would be that if a dominant animal in a feedlot pen gets sick, then other more subordinate animals in the pen may want to fight this animal to achieve higher social status.[2] Bullers should always be checked for signs of disease in addition to being removed from the home pen to prevent severe riding related injuries.[9]

A Canadian study investigated sickness at the time of or shortly after bulling activity.[9] The groups of steers observed in the study were fall steer calves, winter steer calves, and yearling steers. They found 35 % of the fall steer calves, 95 % of the winter steer calves, and 86 % of the yearling steers were recorded as being sick before or at the time of bulling. It was concluded that there is a strong temporal association between bullers and concurrent illness. The risk of disease and mortality in buller steers compared to "normal" pen mates seems to increase with increasing days on feed. Therefore, prompt removal of the buller steer from the pen may lower sickness and mortality in this group. The buller pen requires the same amount of attention concerning pen riding as other higher risk cattle in the feedlot.

Pheromones

Sexually attractive pheromones, supposedly released by buller steers, have been implicated as contributing to the buller syndrome. Pheromones could be contained in the urine and/or feces of the buller steer. A study investigating pheromones analyzed urinary components from ten normal steers and ten buller steers.[2] The urine was assayed colorimetrically for creatinine, 17-ketosteroids, 17-hydroxyketosteroids, and total estrogens

while catecholamines were determined fluroimetrically. The values in Table 2 suggest significant differences between creatinine in the urine of buller steers and normal steers as well as significant differences in 17-hydroxy-corticosteroids between the urine of buller steers and normal steers. The importance of these two compounds in the buller syndrome is not understood. Total urine estrogens didn't differ significantly between the groups.

Table 2. Urinary Parameters of Buller and Normal Steers (Data adapted from Brower et al. 1978)

Urinary components	Bullers (n=10) ± SD	Normals (n=10) ± SD	Probability
Creatinine (g/L)	1.81 ± 0.15	1.33 ± 0.18	$P < 0.1$
Catecholamines (ug/g)	53 ± 13.60	69.41 ± 20.60	ns
Total estrogens (ug/g)	698 ± 97	505 ± 123	ns
17-ketosteroids (mg/g)	13.10 ± 0.00	12.70 ± 3.60	ns
17-hydroxy-corticosteroids (mg/g)	10.30 ± 2.60	3.80 ± 1.26	$P < 0.1$

ns = not significant

Hypothesizing that urine hormones contributed to the buller steer syndrome, researchers applied buller urine, normal steer urine, buller feces, normal steer feces, or water to the tail heads of normal steers.[2] Appling buller urine to the rumps of normal steers resulted in varying reactions, ranging from mounting attempts by other steers to no recognition at all. The application of buller feces to normal steers did not result in mounting attempts. Mounting attempts were made more frequently on steers that had buller urine applied, however, all treatments caused penmate investigation.

Klemm and colleagues hypothesized that if pheromones were not detectable by pen mates, then bulling activity would be decreased.[10] Pheromones are thought to stimulate the olfactory senses through a duct that leads to the vomer nasal organ (VNO). The researchers surgically cauterized the duct that leads to the VNO in yearling steers and did not observe a

reduction in the incidence of buller steers; therefore, pheromones may not play a role in the buller steer syndrome. The act of flehmen was also inconsistent in that study.[10] The results indicate that buller steers probably do not secrete olfactory stimulating substances causing them to be harassed by pen mates.

Exogenous estrogenic substances

The ingestion of exogenous estrogens, i.e. coumestrol, has been implicated in causing buller steers. Coumestrol, a plant estrogen, is found in a variety of forage plants. Bulling activity in steers, prolapsed vaginas, and udder development in heifers are a few adverse effects seen in cattle that consume excessive levels of coumestrol. These effects have been noted when cattle receive an estrogenic implant and are fed a ration consisting of haylage, green chop, or incompletely ensiled silage with coumestrol levels > 37 ppm.[12] Pierson and colleagues observed twice as many bullers in the summer and fall than in the winter and spring, coinciding with feeding alfalfa green chop in the feedyards studied.[7]

Serum hormonal levels

Hormones are chemicals produced by body organs that regulate certain metabolic processes. Estrogen is a steroidal hormone that causes cows and heifers to assume an estrus stance. When steers are observed riding pen mates it is reasonable to speculate that increased serum levels of estrogen may cause type I buller steers.[13] Stress is known to cause release of steroids from either gonads or adrenal glands; either gluccocorticoids or androgens, particularly estrogens may be released.[6,13] If stress is implicated as a cause for steroid hormone release, then special attention should be focused on reducing stress.[14] Table 3 and 4 illustrate the levels of various serum hormones in implanted steers and non-implanted steers.

Table 3. Serum Hormone Levels in Implanted steers

Ref #	Test	Prog	Est	Est 17β	Tren	TA
15						0.29 ppb
15				0.5-0.1 ppb	0.8 ppb	
15						0.15 ppb
15				0.25 ppb		
16		0.38 ng/ml	5.93 pg/ml	1.52 pg/ml		
16			6.87 pg/ml	1.75 pg/ml		
6	43.3 pg/ml			26.9 pg/ml		
17			<1.0-91 pg/ml	<1.0-310 pg/ml		
18	<0.2 ug/l			25ng/l	383 ng/l	
19				14-22.2 pg/ml	676-987 pg/ml	

Test = Testosterone, Prog = Progesterone, Est = Estrone, Est 17β,
Tren = Trenbolone, TA = Trenbolone acetate

Table 4. Serum Hormone Levels in Non-implanted Steers

Ref #	Test	Prog	Est	Est 17β	Tren	TA
20	1.0-4.2 ng/ml					
21	0.09 ng/ml					
15						0.01 ppb
16		0.55 ng/ml	4.98 pg/ml			
22	100 pg/ml					
23	< 500 pg/ml					
23	0.1 ng/ml					
24		0.4 ng/ml		2-4 pg/ml		
16			<1.0-69 pg/ml	<1.0-32 pg/ml		
18	<0.2 ug/l			11ng/L	<25 ng/L	
19				4.2-6.9 pg/ml		

Test = Testosterone, Prog = Progesterone, Est = Estrone, Est 17β,
Tren = Trenbolone, TA = Trenbolone acetate

The results indicate wide ranges in the serum hormone concentrations. A hypothesis might is that hormone concentration vary throughout each day. Irwin and colleagues looked at serum levels of estradiol and testosterone in bullers at the time of bulling and three days post activity in addition to unaffected controls three days post activity.[6] The serum was analyzed using commercially available radioimmunoassay kits. The results of this study are listed in Table 5.

Table 5. Serum Gonadal Hormone Values in Bulling Steers, Determined with Consideration on the Stage of the Affection (Data adapted from Irwin et al. 1979)

	Estradiol mean pg/ml	Testosterone mean pg/ml
While bulling	18.7 (n=26)	21.0 (n=22)
After recovery	25.5 (n=26)	50.9 (n=22)
Unaffected	26.9 (n=7)	43.3 (n=10)

It was found that both serum estradiol and testosterone were significantly lower ($P < 0.01$) in buller steers during bulling than after the recovery period. Wetterman and colleagues found normal steers had higher serum estradiol concentrations than did buller steers.[25] In contrast, Brower and colleagues found higher serum estrogen concentrations in buller steers than normal steers.[2]

There is extensive evidence that differences in serum hormone levels exist between buller and normal steers; however, no particular steroidal hormone has been implicated as the sole reason for buller steer occurrence. Little is known about the riders. Possibly the riders have detectable differences in serum hormone concentrations that cause them to be aggressive. Many studies have focused on buller steers and normal steers, but none have focused on all 3 steer classifications at the same time.

Improper castration

Testosterone levels could also be implicated in causing buller steers because elevated serum androgen levels may increase aggressive sexual behavior such as head butting.[14,25] Improper castration techniques, which may leave part of the testicle in the body, are potential sources of the testosterone. Taylor and colleagues observed 19,170 fall steer calves with 13 % intact bulls (range of 3-28 bulls per pen). They found a small, but significant correlation ($r = 0.27$, $P = 0.04$) between the prevalence of intact bulls in a pen and bullers.[4]

Growth hormone implant effects

It is well recognized that growth hormone implants increase feed efficiency and improve average daily gain in feedlot cattle lowering the cost of production. Many of the growth hormone implants contain various concentrations of estrogenic and androgenic substances. This has led to the hypothesis that the hormones released from these implants may cause bullers.

Treatment of feedlot steers with estrogen resulted in feminization, bulling, and elevated tail heads.[26] Bulling activity occurred 1-3 days post diethylstilbestrol (DES) implantation and continued for 1-2 weeks. Refuting the argument that growth hormone implants are the sole cause of buller steers is the fact that buller steers have been observed in feedlots that do not use growth hormone implants.[26] One small feedyard checked the implant status of six bullers, suspecting they had abscessed or bunched implants. Upon examination it was found that all six bullers had lost their growth hormone implants.[14]

Table 6 below illustrates an association between the annual percentage of bullers and anabolic agent used from 1968-1974.[7]

Table 6. Annual Percentage of Bullers and Anabolic Agent Used, 1968-1974 (Data adapted from Pierson et al. 1976)

Year	Total cattle	# Bullers	% Bullers	Anabolic agent used
1968	264,174	3673	1.39	10 mg DES in feed
1969	292,782	3766	1.27	10 mg DES in feed
1970	359,683	6403	1.78	10 mg DES in feed
Mean %			1.50	
1971	515,885	10,782	2.09	20 mg DES in feed + implant
1972	554,714	15,532	2.80	20 mg DES in feed + implant
1973	431,613	13,639	3,16	20 mg DES in feed + implant
1974	407,450	14,960	3.67	20 mg DES in feed + implant
Mean %			2.88	

DES = Diethylstilbestrol

As demonstrated in this table, from 1968-1970 when DES was fed at a rate of 10 mg/hd/day the percentage of bullers fluctuated from 1.27 to 1.78 % and averaged 1.50 % for the three-year period. During 1971-1974 the annual percentage of bullers increased from 2.09 to 3.67 % and averaged 2.88 %. The increase in bullers between 1971 and 1974 may be due to the transition period going from strict oral feeding of DES in 1971 to oral feeding of DES and growth hormone implantation in 1974.[7] The type of anabolic hormone implants used in 1972-1974 were Synovex-S® and zeranol, a synthetic estrogen like compound (Ralgro®, Schering Plough Animal Health, Union, NJ).

In 1973, Pierson and colleagues evaluated weight gain and feed conversion in cattle implanted with one of 3 implant options; Diethylstilbestrol, Ralgro®, or Synovex-S®. The relationship of bullers to the brand of growth hormone implant administered was recorded.[7] Table 7 illustrates the results of a study.

The progesterone and estrogen combination implant was associated with the greatest incidence of bullers, but this implant produced the most efficient gains. It was used in a minority of cattle in 1972, a majority in 1973, and exclusively in 1974. Irwin and colleagues

also investigated the relationship between growth hormone implants and the percentage of bullers observed. In that study steers were implanted shortly after arrival with one of the following growth hormone implants: Diethylstilbestrol, Ralgro®, or Synovex-S®.[6] Table 8 illustrates the results of that study.

Table 7. Relationship of Bullers to Brand of Implant
(Data adapted from Pierson et al. 1976)

Implant	# Implanted	# Bullers	% Bullers
DES	68,086	1729	2.54
Zeranol [a]	51,216	1123	2.19
Progesterone + estradiol benzoate [b]	42,020	1691	4.02

DES = Diethylstilbestrol, [a] = Ralgro®, [b] = Synovex-S®

Table 8. The Occurrence of Bulling in Relation to Implant Used
(Data adapted from Irwin et al. 1979)

Implant	% Bullers	No. Implanted
Progesterone + estradiol benzoate [a]	2.46	13,244
DES	1.37	5463
Zeranol[b]	0.46	1721

DES = Diethylstilbestrol, [a] = Synovex-S®, [b] = Ralgro®

Treatment with DES alone was associated with significantly fewer bullers ($P < 0.001$) than the Synovex-S® implant.[6] Another comparison of implants was conducted by Booker and colleagues.[27] The first treatment consisted of Ralgro® at allocation followed by administration of a combination of 24 mg estradiol 17β and 120 mg trenbolone acetate implant (Revalor -S®, Intervet, Sommerville, NJ) at day 70 of the feeding period. The second treatment group was implanted with Ralgro® at allocation followed by a combination implant containing 28 mg estradiol benzoate and 200 mg trenbolone acetate (Synovex Plus ®, Fort Dodge Animal Health, Overland Park, KS) at day 70 of the feeding period. The third

treatment group was implanted with Synovex Plus® at allocation only. The mean days on feed for each treatment was 147 days. The percentages of initial rider rate, first rider relapse, and second rider relapse were also recorded in each treatment group. Table 9 reports the results of the initial rider and relapse rates by implant treatment for this trial.

Table 9. Initial Rider and Relapse Rates by Implant Program (Data adapted from Booker et al. 1997)

Occurrence	Treatment A	Treatment B	Treatment C	SE
Initial rider rate %	3.99 [a]	5.06 [a]	9.93 [b]	0.64
First rider rate %	42.23 [a]	39.03 [a]	44.67 [a]	4.27
Second rider rate %	49.96 [a]	49.87 [a]	45.67 [a]	4.72

Treatment A- Ralgro® at allocation followed by Revalor-S® 70 days later.
Treatment B- Ralgro® at allocation followed by Synovex-Plus® 70 days later.
Treatment C- Synovex-Plus® at allocation with no re-implantation.
[a b] Means in a row with different superscripts are significantly different ($P < .05$)

From the results of this trial it appears that a higher potency implant given on arrival may cause increased buller activity. The type of trenbolone acetate implant given at re-implantation had no significant effect on the number of bullers.[27] Implants haven't been implicated as the sole reason buller steers occur. However, practices that allow hormones in the implant to release faster or slower than normal may cause variation of serum hormone levels in a pen population, which may contribute to the buller steer syndrome. Those improper practices include placement of the implant too close to the head, bunching implants, and poor techniques that result in abscessed implants. Administering lower potency implants on arrival before steers receive terminal trenbolone acetate implants may result in fewer bullers, therefore, strategic implant programs must be developed by the veterinarian and producer.

Social behavior effects

It has been suggested that captivity is a more powerful agent of behavioral change than might be imagined, and that captivity or confinement may result in boredom, invasion of personal space, and ritualized games.[28] One hypothesis is that buller steers and riders are responding to the confined feedlot environment they are placed into; under pasture or range conditions the buller steer is very rare.[26]

The most likely practice to be imposed on feedlot cattle after arrival is the mixing and confinement of unfamiliar cattle into pen groups. Antagonistic interactions occur as these cattle establish a social hierarchy. Studies have shown the peak incidence of bullers occurs in the immediate post-arrival period, soon after unfamiliar cattle from many sources are co-mingled into pen groups.[4] Most buller cases occur within the first 30 days on feed.

In a normal free ranging herd, the males are socially dominant over females and mounting may be a primary cue by which females learn to accept that dominance. The least dominant cattle in a herd setting tend to avoid social interactions.[28] Bullers may not readily submit to dominance by pen mates, thereby posing a continuous challenge to others in the pen. Repeated mounting rituals may be an attempt by steers trying to impose social dominance on pen mates.[10]

Lott and colleagues recorded buller behavior in an 11-year study of American bison bulls. They found that the buller behavior occurred in calves and yearling bulls, increased in frequency among 2-3 year olds, declined among 4 year olds, and was virtually absent among 5 year olds.[3] Even though bulls were evaluated, the study may suggest that the buller syndrome may be normal behavior in young feedlot steers. As table 1 illustrated, the incidence of buller steer occurrence declines with increasing age.

Attempted therapies

Various therapies used in the past were based on the theory of a pheromone etiology; the therapies were used to mask the buller odor. Agents such as screw worm spray, fish oil, and ammonia have been applied to the buller. Feedlot personnel suggest these are useful in some bullers, but others report that without treatment 70 % of first occurrence bullers that are re-introduced to the home pen are never removed again.[14,29]

Management

Many of the suggestions for management of bullers are based on anecdotal information, experience, and in some cases logic. As mentioned previously, stress should be minimized especially during the receiving period. Avoiding overcrowding pens of cattle, providing adequate water and bunk space, and especially avoid excessive re-grouping either on arrival or during re-implantation may help decrease the incidence of bullers.[14]

It has been speculated that errors in bunk management contribute to buller activity by causing boredom and subclinical acidosis. Cattle may increase aggressive acts in response to boredom. Cattle that are depressed because of subclinical acidosis may be the recipients of harassment by other pen mates that want to challenge the social status in the pen. Quality of implant placement may also play a role in buller activity; however, implants have not been implicated as the main cause of buller steers. If implants are crushed, abscessed, bunched or missing, this could cause variation in levels of serum hormones throughout the pen. Research suggests that this variation may be a reason steers are prone to bulling; therefore, scheduled implant monitoring should be considered routine in a feedlot. Re-implanting earlier than suggested (stacking) may also increase levels of serum hormones.[14]

Buller cages in the pen may provide some relief to the buller, but this should not take the place of removing them from the pen. A buller cage is a small metal structure typically placed on the fence. The steer can then walk under the cage to prevent harassment by pen mates. Early removal of the buller from the pen decreases the injury to the buller while increasing the likelihood of successful re-introduction. One practical way of re-introduction is to remove 10-15 head from the home pen into a drover's alley where the buller is located. After the group is mixed, then return the entire group back to the pen. Re-introduction at re-implantation time has also been successful.[14]

Summary

Factors related to incidence of the buller syndrome include, but are not limited to, seasonality, pen size and density, group mixing, concurrent disease, pheromones, exogenous estrogens, serum steroid hormone level, improper castration, growth hormone implant effect, and social interactions.[2,3] No specific causative factor has been implicated as the sole reason for the occurrence of buller steers. The above factors may exert an influence independently or in combination. Conflicting reports of the serum hormone status of buller and normal steers have been investigated, but little is known about the serum hormone status of the rider at the time of bulling activity. Further research is needed to determine whether the buller or rider is responsible. Applying and adhering to good management practices at this time is the best way to minimize losses from buller steers.

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CHAPTER 2. COMPARISON OF SERUM STEROIDAL HORMONE CONCENTRATIONS IN BULLER STEERS, RIDERS, AND UNINTERESTED PENMATES: IMPLICATION FOR THE ETIOLOGY OF THE BULLER STEER SYNDROME IN NORTH AMERICAN FEEDLOTS

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Summary

The following parameters were recorded in rider steers (n = 17), buller steers (n = 6), and control steers (n = 18) at the time of bulling activity: body weight on day 1, rectal temperature on day 1 and 3, implant condition on day 1, and serum hormone concentrations of trenbolone, trenbolone acetate, testosterone, progesterone, and estradiol 17β on day 1 and 3. Day 1 was considered the day of initial bulling activity. The data was analyzed for differences among the previously mentioned parameters between the steer groups.

Analysis of the continuous variables found body weight at the time of bulling did not differ between groups (P = 0.99). The rectal temperatures at the time of bulling did not differ between groups (P = 0.93), and the rectal temperatures on the third day post bulling activity did not differ between groups (P = 0.80). The relationship between body weight at the time of bulling activity and day 1 rectal temperature was significant (P = 0.002). The relationship between body weight at the time of bulling and day 3 rectal temperature was not significant (P = 0.31).

Analysis of the categorical variables found that the condition of growth hormone implants at the time of bulling did not differ between groups (P = 0.27). The difference in day 1 estradiol 17β concentration between steer groups was significant (P = 0.05), and 4/4 steers with detected quantified concentrations of estradiol 17β on day 1 were riders. One

buller and 1 control had detected-not quantified levels on day 1. The level of detection and level of quantification for estradiol 17β was 1ppb and 10 ppb respectively. The results suggest the rider may have elevated levels of estradiol 17β in relation to the bullers and non-involved penmates at the time of bulling activity and further research concerning the status of the rider is warranted.

Introduction

A buller steer is defined as a steer that is relentlessly ridden and harassed by a group of pen mates. The average incidence reported for buller steers in a feedlot population is 2-3 % (range 0-11.2 %), and the case fatality rate may exceed 1 %.[1-3] A survey ranked the buller steer syndrome third behind bovine respiratory disease and foot rot as the most costly disease in North American feedlots.[1] It is estimated that buller steers cost the cattle industry $70 per head. Death loss, carcass condemnations, decreased live weight gain, and treatment of injury cause economic loss.

Buller steers have been classified as either type I or type II.[4] Type I buller steers are considered the "true buller." These steers assume a stance similar to what is seen when pubertal heifers are in estrus. It is not uncommon for these steers to be ridden and harassed to the point of collapsing. Type II buller steers are considered steers of "unfair social circumstances," and these buller steers will not assume an estrus like stance. The type II buller steer will use aggressive acts such as head butting to ward off the group of riders. Eventually the buller steer succumbs to the harassment and lies down; however, the riders will continue their activities on the downed buller steer.

Proposed causes of the buller syndrome include, but are not limited to, season, pen size and density, group mixing, concurrent disease, pheromones, exogenous estrogens, serum

steroidal hormone concentration, improper castration, growth hormone implant effect, and social interactions.[1,2] These factors may exert an influence independently or in combination. To date, no specific causative factor has been implicated as the sole reason for the buller steer syndrome.

The aim of the study was to determine if there is a difference in serum concentrations of trenbolone, trenbolone acetate, testosterone, progesterone, and estradiol 17β in buller steers, riders, and uninterested pen mates on the day of bulling activity and 3 days post activity.

Materials and Methods

Cattle

A retrospective case-control observational study was performed from September 15, 1999 through November 30, 1999 at a 4000 head feedlot in southwest Iowa. The parameters investigated were weight at the time of bulling, rectal temperature on day 1 and 3, bunk score at the time of bulling, condition of growth hormone implant at the time of bulling, and serum hormone concentrations on day 1 and 3. Day 1 was considered the day of initial bulling activity. All steers in the yard during this time period were eligible for sampling. The steers were housed in open dirt lots with excellent slope and access to shade. Steers were fed twice a day using a fence line concrete bunk with an 8-foot apron. The feedlot manager was responsible for feed allocation. The ration consisted of corn gluten, whole shell corn, haylage, corn silage, and a protein supplement.

The steers sampled were yearlings originating from multiple sources in the Midwest. Lot 126, tag numbers 1-127, arrived on July 21, 1999, and tag numbers 128-188 arrived six days later on July 27, 1999. All steers were processed within 24 hrs after arrival.

Lot 126 received an intramuscular modified live virus (MLV) vaccine for Infectious Bovine Rhinotracheitis Virus (IBR), Bovine Viral Diarrhea Virus (BVD), Bovine Respiratory Syncitial Virus (BRSV), Parainfluenza 3 Virus (PI3), a *Haemophilus somnus* bacterin, intranasal MLV vaccine (IBR, PI3), systemic avermectin, a multivalent clostridial bacterin, and were implanted with a combination 120mg trenbolone acetate/24mg estradiol product (Component TE-S®, Vet Life, Winterset, IA). The average weight at processing for Lot 126 was 778 lbs. Lot 128, tag numbers 1-150, arrived on September 4, 1999. These steers received the same intramuscular MLV respiratory vaccine (IBR, BVD, BRSV, PI3), systemic avermectin, and were implanted with Component TE-S®. The average in-weight at processing for steers in Lot 128 was 851 lbs.

Management of cases

Pens were checked 3-4 times a day from 6 am-8 pm for buller activity. Tag numbers were recorded for the buller, 3 riders, and 3 uninterested pen mates while bulling activity occurred, prior to removal from the home pen. The entire group was taken to the hospital facility for evaluation.

A hydraulic chute was used to restrain the steers while the weight was recorded. The rectal temperature was recorded at this time using a GLA® thermometer (GLA Agricultural Electronics, San Luis Obispo, CA). If the rectal temperature was $\geq$ 104°F, then other disease processes were noted and treated. The condition of the implant was recorded as being good, missed, or abscessed. A halter was used to restrain the steer's head while 24 mls of venous blood was collected from the jugular vein into 2-12 ml glass tubes using a sterile 18-gauge 1.5-inch Monoject® blood collecting system (Sherwood Medical, St. Louis, MO). The tubes were allowed to clot at room temperature and were subsequently centrifuged for 10 minutes.

The serum was poured into labeled plastic falcon tubes and frozen (-160°F) pending laboratory analysis. Senior veterinary students were responsible for data and sample collection.

After examination, the buller was separated from the riders and uninterested pen mates and all steers were kept in hospital pens until reevaluation on day 3. The group was not placed into pens containing compromised cattle. The same procedures that were performed on day 1 were repeated on day 3. All steers were returned to the home pen following the final procedure.

Analytical Methods

Preparation of corticosteroid standards

Ten milligrams of testosterone, trenbolone, trenbolone acetate, progesterone, and estradiol 17β were weighed individually using an analytical balance. Each steroidal hormone was placed into labeled 10 ml volumetric flasks and 10 ml of reagent grade acetone was added. The 10 ml flask was then vortexed for 10 seconds. The 10 ml solution was removed and placed into a 15 ml glass screw cap tube. The tube was capped with a lid, and labeled with the hormone, the date, and the concentration (1mg/ml).

A stock solution was then developed for use as the standard for steroid hormone analysis. Twenty microliters of each steroid hormone solution was placed into a 10 ml volumetric flask. Ten milliliters of reagent grade acetone was then added to the flask, capped with a lid, and vortexed for 10 seconds. The solution from the flask was then removed and placed into a 15 ml glass screw cap tube. The tube was capped with a lid, labeled with the names of the standards in the mix, and the date completed. The standards were placed into a cooler maintained at -13°F.

Extraction of corticosteroids

The serum samples were removed from the freezer and allowed to thaw at room temperature. The serum samples were vortexed for 10 seconds, and 1 ml aliquots of serum from day 1 and 3 were placed into individually labeled 15 ml glass screw cap tubes. The 1 ml sample was considered the test sample. Due to inadequate serum volume only 0.5 ml aliquots of serum from day 1 and 3 were placed into similar individually labeled 15 ml tubes. One hundred microliters of the standard hormone stock solution was added to the 0.5 ml sample. The 0.5 ml sample was considered the spiked control. Five milliliters of diethyl ether was added to each tube and centrifuged for 20 minutes at 2000 RPM. The samples were removed from the centrifuge and the diethyl ether fraction was removed by passing it through a sodium sulfate column into a two-dram glass vial.

The diethyl ether solution contained in the two-dram glass vials were desiccated using nitrogen gas effusion. One hundred microliters of a 60:40 methanol:milipore water solution was added to the glass vials to re-solvate the hormone content, and these solutions were vortexed for 10 seconds. The samples (4 per steer) were analyzed using YMC C18 reverse phase high performance liquid chromatography (HPLC) with a 5 micron size separation and 4.6 x 150 column. The HPLC operator was blinded to steer classification. The levels of detection (LOD) and levels of quantification (LOQ) are listed in Table 1.

Table 1. Level of Detection and Quantification of Serum Hormones in Study

Hormone	Level of Detection (ppb)	Level of Quantification (ppb)
Testosterone	0.2	2
Trenbolone	0.2	2
Trenbolone acetate	0.5	5
Progesterone	1	10
Estradiol	1	10

Statistical analysis

Data from steers in buller (n = 6), rider (n = 17), and control (n = 18) groups were evaluated. Day 1 weight, and day 1 and day 3 rectal temperature were analyzed as continuous variables. Due to the uneven distribution of steer classification, the continuous variables were analyzed using the general linear model procedure in JMP 4.0.2® (SAS Institute Inc. Cary, NC).

Serum hormone concentration and implant condition were analyzed as categorical variables. The condition of the growth hormone implant at the time of bulling activity were classified as good, abscessed, or missed. The serum hormone concentrations on day 1 and day 3 were categorized as non-detected, detected-not quantified, and detected-quantified variables. Serum hormone concentrations were analyzed as categorical variables because very few samples (7/350) had detected quantified hormone concentrations; therefore, analysis of variance would not be adequate because of the high number of "zero" values.

Trenbolone acetate and progesterone hormone concentrations on day 1 and day 3 in all steer groups were non-detected; therefore, those hormones were not included in the analysis. Those steers with inadequate serum volume were also not included in the categorical variable analysis. The hormone classes used in the statistical analysis were day 1 and day 3 trenbolone, day 1 and day 3 testosterone, and day 1 and day 3 estradiol 17β.

For analysis of categorical variables the Fishers Exact Test was used in SAS 8.0® (SAS Institute Inc. Cary, NC). The Fishers Exact Test was applied instead of the Chi-square test because expected frequencies were less than 5 in any one cell. A significance level of 5% was used in the analysis for both continuous variables and categorical variables.

Results

One steer group was from Lot 128, which had 10 days on feed at the time of bulling activity. Five steer groups came from Lot 126, and the average days on feed at the time of bulling activity was 67 days with a range of 50-87 days. Feed bunk scores were recorded at the time bullers were identified. Five of the 6 steer groups had a feed bunk score of 0 (empty) and 1 group had a feed bunk score of 2 (one-fourth of ration left in bunk). The majority of bullers (5/6) in this study were identified while feed bunks were empty.

The number of steers per classification, mean, range, and standard deviations of the weight on day 1, rectal temperature on day 1 and day 3 are listed in Table 2.

Table 2. Day 1 Weight (pounds), Day 1 and Day 3 Rectal Temperature (°F) of Riders, Bullers, and Controls

	Rider (n=17)	Buller (n=6)	Control (n=18)
WT-1 mean ± SD	1107 ± 178	1104 ± 128	1111 ± 168
WT-1 range	806-1404	924-1286	802-1406
TP-1 mean ± SD	103.1 ± 1.1	103.3 ± 1.6	103.1 ± 0.9
TP-1 range	101.4-105.3	101.1-105.3	101.9-104.9
TP-3 mean ± SD	102.6 ± 0.7	102.8 ± 1.3	102.6 ± 0.8
TP-3 range	101.2-103.9	101.6-105.1	101.0-104.0

WT-1 = Day 1 Weight, TP-1 = Day 1 Rectal Temperature,
TP-3 = Day 3 Rectal Temperature, SD = Standard Deviation

Continuous variable analysis

Body weight at the time of bulling activity did not differ between steer groups (P=0.99). Rectal temperature on day 1 and 3 did not differ between steer groups (P = 0.93 for day 1, P = 0.80 for day 3). The difference between day 1 rectal temperature and day 3 rectal temperature was not significant (P = 0.20). The relationship between day 1 rectal temperature and body weight at the time of bulling activity was significant (P = 0.002).

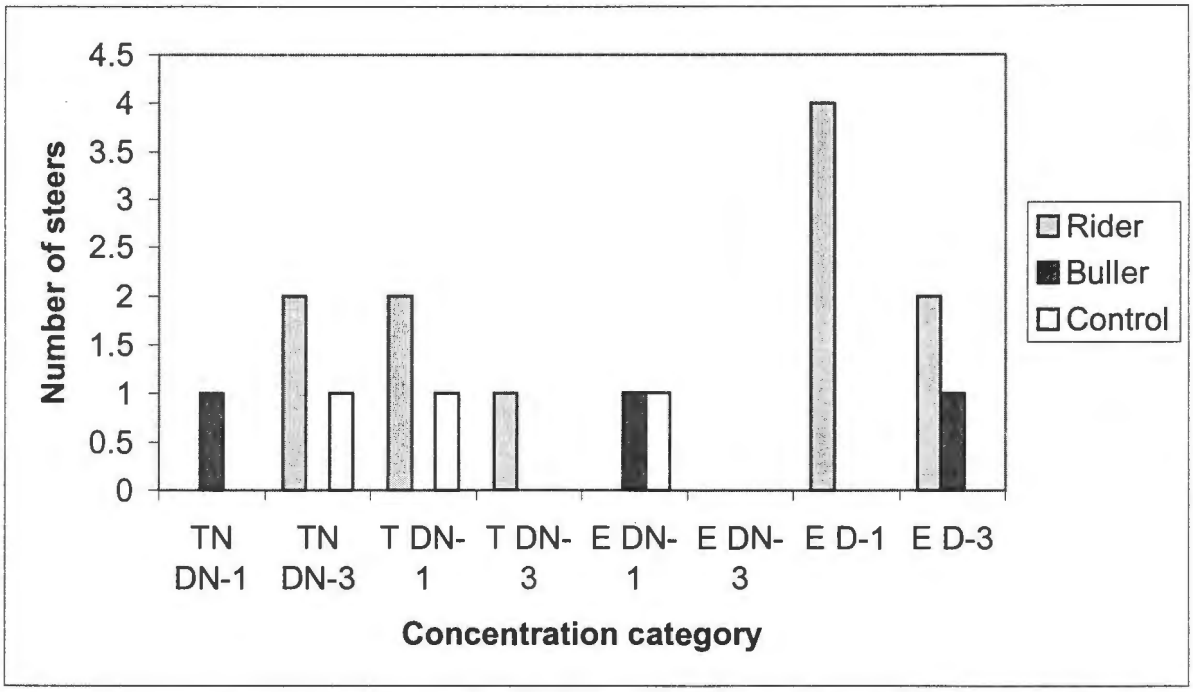
Categorical variable analysis

Thirty-eight steers were evaluated for day 1 serum trenbolone concentrations. Trenbolone concentrations in 1 buller were detected-not quantified on day 1. The serum concentration of trenbolone on day 1 did not differ between steer groups (P = 0.14). Thirty-two steers were evaluated for day 3 serum trenbolone concentrations. Trenbolone concentrations in 2 riders and 1 control were detected-not quantified on day 3. The serum concentration of trenbolone on day 3 did not differ between steer groups (P = 0.45).

Thirty-eight steers were evaluated for day 1 serum testosterone concentration. Testosterone concentrations in 2 riders and 1 control steer were detected-not quantified on day 1. The serum concentration of testosterone on day 3 did not differ between steer groups (P = 0.74). Thirty-two steers were evaluated for day 3 serum testosterone concentrations and 1 rider had detected-not quantified concentrations of testosterone. The serum concentration of testosterone on day 3 did not differ between groups (P = 1.0).

Thirty-eight steers were evaluated for day 1 serum estradiol 17β concentrations. Estradiol 17β concentrations in 1 control steer and 1 buller were detected-not quantified on day 1. Estradiol 17β concentrations in 4 riders were detected-quantified on day 1. The serum concentration of estradiol 17β on day 1 was significantly different among steer groups (P = 0.05). Thirty-two steers were evaluated for day 3 serum estradiol 17β concentrations. Estradiol 17β concentrations in 2 riders and 1 buller were detected-quantified on day 3. The serum concentration of estradiol 17β on day 3 did not differ between steer groups (P = 0.38). The hormone concentration categories and the number of bullers, riders, and controls in each category are demonstrated in Figure 1.

Figure 1. Number of Steers Per Hormone Concentration Category



TN DN-1 = Trenbolone detected not quantified on day 1, TN DN-3 = Trenbolone detected not quantified on day 3, T DN-1 = Testosterone detected not quantified on day 1, T DN-3 = Testosterone detected not quantified on day 3, E DN-1 = Estradiol 17β detected not quantified on day 1, E DN-3 = Estradiol 17β detected not quantified on day 3, E D-1 = Estradiol 17β detected quantified on day 1, E D-3 = Estradiol 17β detected quantified on day 3

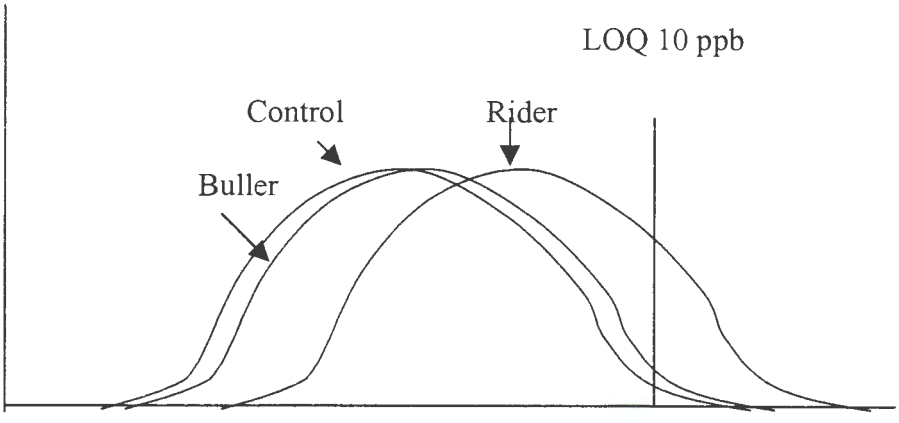
Three control steers had missing growth hormone implants, and one rider had an abscessed growth hormone implant. The steers with missed and abscessed growth hormone implants did not have detectable serum hormone concentrations. The remaining steers had good growth hormone implants. The relationship between growth hormone implant condition and steer classification was not significant ($P = 0.27$). The buller steers ($n = 6$) were returned to their home pen after data collection and none were reclassified as a buller during the 10-week study period. Only 1 rider and 2 control steers were re-pulled later in other groups.

Discussion

Day 1 estradiol 17β was the only serum hormone that was significantly different among the steer groups ($P \leq 0.05$). The LOD and LOQ for estradiol 17β in this study were 1 ppb and 10 ppb respectively, which is not as sensitive as previous serum hormone studies.[5] A lower LOQ was not achieved because of low serum sample volume. The volume of serum for each steer was divided and frozen for possible repeat analysis. A lower LOQ would have been possible, but if errors occurred during extraction, then serum would not be available for repeat analysis.

The study results indicate 86 % (6/7) of the steers that had detected quantified concentrations ($\geq$ 10 ppb) of estradiol 17β either on day 1 or 3 were riders. If hormone concentrations in riders, bullers, and control steers are normally distributed in a feedlot population, then one hypothesis would be that rider steers normally have elevated estradiol 17β concentrations compared to bullers and controls. Figure 2 demonstrates the hypothesis drawn from this study.

Figure 2. Serum Hormone Concentration Hypothesis in Riders, Bullers, and Controls



A relationship was found between day 1 rectal temperatures and body weight at the time of bulling activity. This is probably related to the effect of environmental temperatures and activity (transport to hospital facility) on heavier cattle; therefore, heavier cattle may have higher normal body temperatures. The cattle in each classification that had rectal temperatures $\geq$ 104.0° F exhibited no evidence of clinical disease. The data suggests disease was not a cause for initiating bulling activity in this study. Weight at the time of bulling also had no effect on the incidence of bulling.

This is the first study to examine serum hormone concentrations in riders as well as bullers and uninterested pen mates. Although the assay level of detection used was of low sensitivity, the results of this study suggest that the rider should be scrutinized as closely as the buller in future studies.

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CHAPTER 3. GENERAL CONCLUSIONS

The research presented in this thesis establishes the groundwork for further investigations into serum hormone concentrations of rider steers and their implications for contributing to the buller steer syndrome in North American feedlots. Significant differences ($P = 0.05$) were demonstrated in serum estradiol 17β concentrations at the time of bulling activity. The 4 steers that had serum concentrations of estradiol 17β above the assay level of quantification were all classified as riders. The level of quantification for estradiol 17β in this study was not as sensitive as other studies, but the available data support the hypothesis that the rider steer has elevated estradiol 17β at the time of bulling activity as compared to the buller and uninterested pen mates. The results of this study suggest that the rider should be scrutinized as closely as the buller in future studies.

APPENDIX A. RAW DATA

ID	TN-1	TN-3	T-1	T-3	TA-1	TA-3	P-1	P-3	E-1	E-3	Class	WT	TP-1	TP-3	IC
128-23	N	T	N	T	N	N	N	N	N	N	R	832	102.4	103.3	G
128-49	N	N	N	N	N	N	N	N	N	N	R	806	102.3	103.9	G
128-39	N	N	N	N	N	N	N	N	N	N	C	920	102	101.5	G
128-72	N	N	N	N	N	N	N	N	N	N	C	924	102.4	102.1	G
128-125	N	N	N	N	N	N	N	N	N	N	C	802	102.3	101	G
128-136	N	N	N	N	N	N	N	N	N	N	B	924	101.1	101.6	G
126-10	N		N		N		N		N		C	996	102	103	G
126-22	N		N		N		N		N		C	1084	101.9	102.3	G
126-5	N		N		N		N		56		R	1062	102.2	102.6	G
126-1	N		N		N		N		28		R	1124	102	102.3	G
126-186	N		N		N		N		N		C	1066	103.2	103.3	G
126-45	N		N		N		N		N		R	930	103.2	101.2	G
126-168											B	1046	101.8	103.6	G
126-22	N	N	N	N	N	N	N	N	N	N	C	1110	102.4	102.6	G
126-30	N	N	N	N	N	N	N	N	N	N	C	896	103	101.4	G
126-54	N	N	N	N	N	N	N	N	N	N	C	1084	103.3	102.7	G
126-116	N	N	N	N	N	N	N	N	N	N	R	918	101.4	102	G
126-68	N	N	T	N	N	N	N	N	N	N	R	1166	102.9	102.9	G
126-148	N	N	N	N	N	N	N	N	10	18	R	1060	102.1	102.4	G
126-8	N	N	N	N	N	N	N	N	N	23	B	1036	104.2	102.6	G
126-118	N	N	T	N	N	N	N	N	N	N	R	934	104.1	102.5	G
126-187	N	N	N	N	N	N	N	N	N	N	C	1138	104.1	103.4	G
126-125	N	N	N	N	N	N	N	N	17	10	R	1202	104.4	102.2	G
126-157	T	N	N	N	N	N	N	N	N	N	B	1186	103.9	102	G
126-27	N	T	N	N	N	N	N	N	N	N	R	1252	105.3	102.7	G
126-9	N	N	N	N	N	N	N	N	N	N	C	1284	104.9	103.8	G
126-179	N	N	T	N	N	N	N	N	N	N	C	1208	103.4	102.4	G
126-160	N	N	N	N	N	N	N	N	N	N	R	1248	103.2	101.6	G
126-49	N	N	N	N	N	N	N	N	N	N	C	1184	103.5	101.9	M
126-96	N	N	N	N	N	N	N	N	N	N	C	1276	103.7	102.7	G
126-120	N	N	N	N	N	N	N	N	T	N	C	1010	103.1	103.1	M
126-90	N	N	N	N	N	N	N	N	N	N	R	1050	103.9	102.2	A
126-27	N	N	N	N	N	N	N	N	N	N	R	1288	102.6	102.3	G
126-134	N	N	N	N	N	N	N	N	N	N	B	1150	103.5	102	G
126-166	N	N	N	N	N	N	N	N	N	N	C	1406	102.9	102.4	G
126-164	N	N	N	N	N	N	N	N	T	N	B	1286	105.3	105.1	G
126-28	N	N	N	N	N	N	N	N	N	N	C	1332	104.4	102.9	M
126-86											R	1342	105	103.4	G
126-187											C	1282	104	104	G
126-103	N	N	N	N	N	N	N	N	N	N	R	1404	103	103.1	G
126-15	N	N	N	N	N	N	N	N	N	N	R	1204	102.8	102.8	G

The abbreviations for the table are as follows: TN-1 = Day 1 Trenbolone, TN-3 = Day 3 Trenbolone, T-1 = Day 1 Testosterone, T-3 = Day 3 Testosterone, TA-1 = Day 1 Trenbolone Acetate, TA-3 = Day 3 Trenbolone Acetate, P-1 = Day 1 Progesterone, P-3 = Day 3 Progesterone, E-1 = Day 1 Estradiol 17E-2 = Day 3 Estradiol 17β, N = Not Detected, T = Detected-not quantified, TP-1 = Day 1 Rectal Temperature, TP-3 = Day 3 Rectal Temperature, M = Missed implant, A = Abscessed implant, G = Good implant, R = Rider, B = Buller, C = Control.